

G. Finazzi  
T. Barbui<sup>1</sup>

Divisione di Ematologia e  
<sup>1</sup>Fondazione per la Ricerca  
Ospedali Riuniti di Bergamo,  
Italy

## Hydroxyurea: the comparator in studies with new anti-JAK2 inhibitors



The fundamental discovery of V617F and other mutations in the JAK2 gene of most patients with Philadelphia-negative chronic myelo-proliferative neoplasms (MPNs) has changed the diagnostic approach to these disorders and opened the way to a molecularly-targeted therapy.<sup>1-6</sup> A new generation of drugs with more or less specific inhibitory activity against JAK2 has been developed and it is now in advanced stages of clinical evaluation.<sup>7</sup> As a rule, the efficacy and safety of a new therapeutic agent in a given disease should be compared with the best available treatment in randomized phase III clinical trials. In the most frequent MPNs, that are essential thrombocythemia (ET) and polycythemia vera (PV), the standard myelosuppressive therapy for patients with a high risk of thrombosis is hydroxyurea (HU). This drug is also the first choice for patients with myelofibrosis (MF) and symptomatic splenomegaly or requiring cytoreduction. The purpose of this chapter is to review the expected benefits and risks of HU in patients with MPNs in order to establish the bottom line for comparative studies with new drugs.

### Chemistry and mechanism of action

HU ( $\text{CH}_4\text{N}_2\text{O}_2$ , Figure 1) is FDA-approved as an anti-neo-

plastic agent in treatment of MPNs and other cancers, such as melanoma, and also for reducing the frequency of painful crises and the need for blood transfusions in patients with sickle cell anemia (SCA). HU is not recommended for use during pregnancy, although occasional cases of HU therapy during pregnancy have been reported.<sup>8</sup> HU inhibits the enzyme ribonucleotide reductase, which catalyzes the conversion of ribonucleotides to deoxyribonucleotides.<sup>9</sup> The depletion of deoxyribonucleotide pools is not complete but is sufficient to inhibit deoxyribonucleic acid (DNA) synthesis, resulting in S-phase cytotoxicity. The cytotoxic effects are believed to be responsible for the utility of HU in treating MPNs. Other mechanisms by which HU therapy may decrease the incidence and severity of thrombosis in ET and PV and of vaso-occlusive crises in SCA include increased nitric oxide production resulting in vasodilatation and reduced platelet aggregation, and reduced neutrophil number with consequent decreases in pro-inflammatory mediators and reduced expression of adhesion molecules.<sup>10,11</sup>

### Efficacy in essential thrombocythemia

Two randomized clinical trials

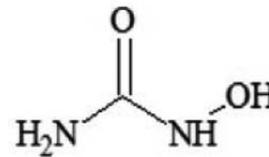
**Table 1.** Randomized clinical trials with HU in ET and PV.

Study (Ref)	Patients and follow-up	Treatments and main results		p
Cortelazzo <i>et al.</i> <sup>12</sup>	114 ET pts; 27 months (median)	HU*	No cytoreduction*	0.003
		2	Thrombosis 14	
Harrison <i>et al.</i> <sup>14</sup>	809 ET pts; 39 months (median)	HU (+ASA)	Anagrelide (+ ASA)	0.004
		17	Arterial Thrombosis 37	
		14	Venous thrombosis 3	
		8	Major bleeding 22	
		5	Transformation to myelofibrosis 16	
		27	Death for any cause 31	
Najean <i>et al.</i> <sup>26</sup>	292 PV pts (age <65 yrs); 16 yrs (max)	HU	Pipobroman	n.s.
		70%	14-year survival 70%	
		17%	Myelofibrosis 2.1%	
		5%**	Acute leukemia 5%**	

ASA=Aspirin; n.s.= not significant; \*plus ASA in 70% of patients in both groups; \*\*after 10 years of follow-up.

assessing HU therapy in ET patients at high risk of thrombosis have been published in full so far (Table 1).

The first was performed in Italy and evaluated HU vs. untreated controls: 114 ET patients were randomized to HU (n=56) or no cytoreductive treatment (n=58).<sup>12</sup> During a median follow-up of 27 months, 2 thromboses (1 stroke and 1 myocardial infarction) were recorded in the HU-treated group (1.6%/pt-yr) compared with 14 in the control group (1 stroke, 5 transient ischemic attacks, 5 peripheral arterial occlusions, 1 deep vein thrombosis, and 2 patients with superficial thrombophlebitis) (10.7%/pt-yr;  $p=0.003$ ). This study provided the basis for considering HU as the standard therapy for high-risk ET patients and the reference arm for other randomized trials. In an extension of this study, the same patients were followed for a median period of 73 months and 5 cases (9%) in the HU group

**Figure 1.** Structure of hydroxyurea.

vs. 26 (45%) in the control group had vascular complications ( $p<0.0001$ ) confirming the antithrombotic value of HU also on the long period.<sup>13</sup> However, no effect on overall survival was seen, since 85% of HU-treated patients vs. 84% controls were alive.

The second trial, named Primary Thrombocythaemia 1 (PT 1), was carried out in UK and compared HU plus aspirin vs. anagrelide plus aspirin in 809 high-risk patients with ET.<sup>14</sup> Anagrelide is an inhibitor of

megakaryocyte differentiation and proliferation, so that it is able to reduce thrombocytosis with minimal or no effect on erythrocyte and leukocyte counts. Overall, patients randomized to anagrelide and aspirin were more likely to reach the composite primary end point of major thrombosis (arterial or venous), major hemorrhage or death from a vascular cause ( $p=0.03$ ). When individual endpoints were assessed arterial thrombosis, major hemorrhage and myelofibrosis were all significantly more frequent for patients treated with anagrelide ( $p=0.004$ ,  $0.008$  and  $0.01$  respectively). Of all categories of arterial thrombosis the difference was only significant for transient ischemic attacks (14 vs. 1), but each were more common in anagrelide treated patients. However anagrelide and aspirin seems to offer at least partial protection from thrombosis, as the prevalence of thrombotic events (8%) was significantly lower than the control arm of the Italian study (28%), while the hydroxyurea arms were approximately equivalent (4% in two years). The success of hydroxyurea is likely to reflect the importance of additional factors such as the hematocrit, leukocyte or neutrophil count, or subtle effects upon the endothelium in the pathogenesis of thrombosis. Intriguingly venous thrombosis was however less frequent in patients treated with anagrelide ( $p=0.006$ ).

In both trials, the doses of HU (and anagrelide in PT1) were titrated to maintain the platelet count below a predefined threshold, that was 600.000/mmc in the Italian and 400.000/mmc in the UK study, respectively. In the PT1 study, control of the platelet count was similar in the two randomized arms by nine months after trial and subsequently. At three and six months after trial entry, platelet counts in the anagrelide group were significantly higher than those in the HU group ( $p<0.001$  for both time points). The median leukocyte count in the HU group was significantly and persist-

ently lower than that in the anagrelide group ( $p<0.001$ ), starting at three months after trial entry. Interestingly, patients enrolled in the PT-1 who were JAK2 V617F-positive required substantially lower doses of HU to reduce their platelet count, leukocyte counts and hemoglobin concentrations than did V617F-negative patients.<sup>15</sup> Furthermore, V617F-positive patients showed particular benefit from HU compared with anagrelide in the reduction of arterial thrombosis.

Another randomized clinical trial comparing HU and Anagrelide (ANAHYDRET study) has been recently completed<sup>16</sup> and it is reported in detail elsewhere in this book.

---

### Efficacy in polycythemia vera

The first group of investigators who studied HU in the management of PV was the Polycythemia Vera Study Group (PVSG). In a paper summarizing their long-term experience, the incidence of thrombosis in 51 patients treated with HU was compared with the incidence of thrombosis in 134 patients treated only with phlebotomy in a previous PVSG trial (01).<sup>17</sup> Therapy with HU was aimed to reduce hematocrit to less than 50%, with only minimal and highly restricted use of supplemental phlebotomy, and platelet count to less than 600.000/mmc. HU was highly effective in decreasing the risk of thrombosis during the first few years of therapy, when the incidence of thrombosis in phlebotomy treated patient is highest. In an analysis conducted at 378 weeks on study, thrombosis was reported in 32.8% of PVSG protocol 01 patients treated with phlebotomy, compared with only 9.8% of patients in the HU group. In a further analysis conducted after 591 weeks (11.4 years) on study, the incidence of thrombosis remained appreciably less in patients given HU than in those treated with phlebotomy alone (25.5% vs. 40.3 %).

Based on these data, the PVSG produced some recommendations for the use of HU in PV patients. Phlebotomy was suggested in all patients to keep the hematocrit below 0.45. Stable patients at low risk for thrombosis might not require additional therapy. In patients at high risk of thrombosis or with a very high phlebotomy requirement, the choice of myelosuppressive agent was age-adapted. Older patients could be managed with 32P, busulfan, or pipobroman; whereas HU was considered the agent of choice in younger patients.<sup>18</sup>

The impact of the PVSG recommendations on the management of PV patients has been assessed in a large, prospective study in Europe (European Collaboration on Low-dose Aspirin in Polycythemia, ECLAP). Although this cohort of patients was not specifically enrolled for establishing the benefit/risk of HU, this survey reports the best available evidence on the expected outcomes of PV patients receiving the current standard of therapy.<sup>19</sup>

The ECLAP study included 1638 patients followed for a median of 2.8 years, 793 of whom (48%) were treated with HU. The target hematocrit and platelet count were below 45% and 400,000/mm<sup>3</sup>, respectively. The proportion of patients below the recommended values after 12 months of follow-up were 48% for hematocrit and 63% for platelet count, respectively. A total of 164 deaths (10%) were recorded for an overall mortality rate of 3.7 per 100 persons per year. As compared with the general Italian population standardized for age and sex, the excess of mortality of PV patients was 2.1 times. Cardiovascular mortality accounted for 41% of all deaths (1.5 deaths per 100 persons per year), mainly due to large vessel arterial events, such as coronary heart disease and nonhemorrhagic stroke. The cumulative rate of non-fatal thrombosis was 3.8 events per 100 persons per year, without difference between arterial and venous thrombosis.

The incidence of cardiovascular complications was higher in patients aged more than 65 years (5.0% patient-year, hazard ratio 2.0, 95% confidence interval [CI] 1.22-3.29,  $p < 0.006$ ) or with a history of thrombosis (4.93% patient-year, hazard ratio 1.96, 95% CI 1.29-2.97,  $p = 0.0017$ ) than in younger subjects with no history of thrombosis (2.5% patient-year). No relationship was found between the rate of thrombosis and the degree of reduction of hematocrit or platelet count.<sup>20</sup>

These figures should be taken into account when a new drug is proposed for reducing the thrombotic complications of PV patients.

---

### Efficacy in myelofibrosis

HU is active in decreasing splenomegaly and marked cytosis in MF and is the most common initial medical therapy used in these patients.<sup>21</sup> Estimated response rate is <50% for splenomegaly, although very little prospective clinical data exists. However, there are several limitations to the use of this agent in MF. First, HU rarely induces a complete resolution of splenomegaly or even a clinical improvement as assessed by the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria, that is >50% improvement sustained for at least 2 months.<sup>22</sup> Nevertheless, more modest reductions in splenomegaly may benefit some patients with MF. A second limitation is that splenomegaly is not as responsive as thrombocytosis to HU and might require a higher dose (i.e. 2-3 grams/day). Third, particularly at higher doses, HU therapy may potentially exacerbate cytopenias, such as anemia.

Interestingly, chemosensitivity to HU is increased in MF and PV patients carrying the JAK2 V617F mutation, as reported above for ET. In a recent study of 69 patients with MF and 56 with PV, HU response in MF, evaluated

according to the IWG-MRT criteria, was significantly and independently associate with the presence of JAK2 V617F (48% vs. 8% response in mutation positive vs. negative cases).<sup>23</sup> In PV, JAK2 V617F allele burden correlated directly with HU response ( $p=0.05$ ) and inversely with daily HU dose in responding patients ( $p=0.02$ ). The mechanisms that are responsible for this phenomenon can only be speculated. It is well known that JAK2V617F occurs at the stem cell level and favors a myeloproliferative phenotype, with skewing toward erythroid proliferation. It is possible that HU, as an antiproliferative drug, is more active in the presence of a higher degree of cell proliferation, and such activity might be even more pronounced in the presence of endogenous myeloproliferation. For the time being, current information suggests that JAK2V617F presence identifies MPNs patients who are likely to respond to HU therapy.

---

#### Drug resistance and adverse effects

Resistance to HU, defined as a failure to reach the expected reduction of elevated blood cell counts, can be observed in a variable proportion of patients with MPNs. However, reliable data on the magnitude of this effect are lacking, mainly due to heterogeneous definitions of “resistance”. To tackle this issue, an international working group was convened to develop a consensus formulation of criteria for defining clinical resistance (and intolerance) to HU in ET (Table 2).<sup>24</sup> A similar process is ongoing in PV. It is expected that these standardizations can help clinicians in establishing the relevance of HU resistance both in clinical trials and in daily practice.

The more frequent side effect of HU is hematopoietic impairment, leading to neutropenia and macrocytic anemia. To control this effect, the recommended starting dose of

**Table 2.** Definition of resistance/intolerance to hydroxyurea in patients with essential thrombocythemia.<sup>24</sup>

- 
- 1) Platelet count higher than  $600 \times 10^9/L$  after 3 months of at least 2 g/day of HU (2.5 g/day in patients with a body weight > 80 kg), or
  - 2) Platelet count higher than  $400 \times 10^9/L$ , and WBC less than  $2,500/\mu L$  at any dose of hydroxyurea, or
  - 3) Platelet count higher than  $400 \times 10^9/L$ , and Hb less than  $10 g/dL$  at any dose of hydroxyurea, or
  - 4) Presence of leg ulcers or other unacceptable mucocutaneous manifestations at any dose of hydroxyurea, or
  - 5) Essential thrombocythemia-related fever.
- 

HU is 15-20 mg/Kg/day followed by a maintenance dose to keep hematocrit and platelet counts at response levels without reducing WBC count values below  $3,000 \times 10^9/L$ . Complete hemogram should be recorded every two weeks during the first 2 months, then every month, and, in steady state in responding patients, every 3 months.<sup>25</sup> Other less frequent, but clinically relevant, adverse effects include mucocutaneous manifestations and drug-related fever (Table 2). Oral and leg ulcers and skin lesions are often disabling for patients and may exacerbate over time or transform into neoplastic lesions. Therefore, their occurrence dictates the withdrawal of the drug.

---

#### Is hydroxyurea leukemogenic?

Some long-term follow-up studies revealed that a proportion of patients with PV or ET treated with HU developed acute leukemia.<sup>17,26,29</sup>

In the PVSG experience with 51 PV patients given HU for a median follow-up treatment of 8.6 years, the incidence of leukemia was 9.8% vs. 3.7% in historical phlebotomized controls.<sup>17</sup> In a randomized clinical trial carried out in France, 292 patients with PV below 65 yrs. were randomized to treatment with HU or pipobroman and followed from 1980 until 1997 (Table 1).<sup>26</sup> Pipobroman is a bromide

derivative of piperazine with a chemical formula similar to the alkylating agents but a mechanism of action also involving metabolic competition of pyrimidine bases. The incidence of secondary leukemia was about 5% at the 10<sup>th</sup> and 10% at the 13<sup>th</sup> year without significant differences between the two groups.

In other studies, however, the use of this drug as the only cytotoxic treatment was rarely associated with secondary malignancies. In an analysis of 25 ET patients younger than 50 years and treated with HU alone for a high risk of thrombosis, no case of leukemic or neoplastic transformation occurred after a median follow up of 8 years (range 5-14 years).<sup>27</sup> The leukemic risk of HU in PV was evaluated in the 1,638 patients prospectively enrolled in the ECLAP study, with a median disease duration of 6.3 years. HU alone did not enhance the risk of leukemia in comparison with patients treated with phlebotomy only (hazard ratio 0.86, 95% CI 0.26-2.88;  $p=0.8$ ) whereas this risk was significantly increased by exposure to radiophosphorus, busulphan or pipobroman (hazard ratio 5.46, 95% CI 1.84-16.25;  $p=0.002$ ). The use of HU in patients already treated with alkylating agents or radiophosphorus also enhanced the leukemic risk (hazard ratio 7.58, 95% CI 1.85-31;  $p=0.0048$ ).<sup>28</sup>

Other Authors confirmed that HU is associated with a more frequent progression to AL when given before or after alkylating agents or radiophosphorus. In a long-term follow-up study of 112 ET patients, none of 20 patients never treated with chemotherapy developed neoplasia, as compared with 3 of 77 given HU only (3.9% n.s.) and 5 of 15 given busulfan plus HU (33%  $p<0.0001$ ).<sup>13</sup> Sterkers *et al.* reported 14% rate of leukemia when HU was combined with other cytotoxic agents, generally pipobroman.<sup>29</sup> Six cases of AML (21%) out of 28 ET patients treated with HU plus alkylating agents or radiophosphorus were observed by Murphy *et al.*<sup>30</sup>

Two studies from France<sup>29</sup> and Italy<sup>31</sup> revealed a high frequency of 17p chromosomal deletions in patients with acutely transformed disease who were treated with HU, suggesting that these cytogenetic abnormalities might represent a possible leukemogenic mechanism of the drug. However, the 17p deletion also occur in other hematological disorders, including both de novo and treatment-related cases of AL and myelodysplastic syndromes.

To date there are no randomized studies powered to assess the relative risk of malignant transformation in HU-treated patients both in ET and PV. These disorders have an inherent tendency to evolve into AL, even in the absence of specific therapy. Thus, studies that enrolled patients in need of therapy automatically selected patients with more active disease and thus with a higher propensity to malignant transformation. Furthermore, leukemic transformation occurs after a lead-time of several years. In conclusion, the bulk of evidence does not support a clear leukemogenic role for HU. Nevertheless, a cautionary principle suggest to consider carefully the use of this agent in very young subjects and in those carrying cytogenetic abnormalities or previously exposed to radiophosphorus or alkylating drugs.

---

## References

1. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* 2008;22:14-22.
2. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005;365:1054-61.
3. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005;7:387-97.
4. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005;434:1144-8.
5. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005;352:1779-90.
6. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocy-

- tosis. *N Engl J Med* 2007;356:459-68.
7. Pardanani A. JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. *Leukemia* 2008; 22:23-30.
  8. Liebelt EL, Balk SJ, Faber W et al. NPT-CERHT expert panel report on the reproductive and developmental toxicity of hydroxyurea. *Birth Defects Res* 2007;80:259-366
  9. Koc A, Wheeler LJ, Mathews CK, Merrill GF. Hydroxyurea arrests DNA replication by a mechanism that preserves basal dNTP pools. *J Biol Chem* 2004;279: 223-30.
  10. Halsey C, Roberts IA. The role of hydroxyurea in sickle cell disease. *Br J Haematol* 2003;120:177-86.
  11. Maugeri N, Giordano G, Petrilli MP et al. Inhibition of tissue factor expression by hydroxyurea in polymorphonuclear leukocytes from patients with myeloproliferative disorders: a new effect for an old drug? *J Thromb Haemost* 2006;4:2593-8.
  12. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea in the treatment of patients with essential thrombocythemia at high risk of thrombosis: a prospective randomized trial. *N Engl J Med* 1995;332:1132-6.
  13. Finazzi G, Ruggeri M, Rodeghiero F, Barbui T. Second malignancies in patients with essential thrombocythemia treated with busulphan and hydroxyurea: long-term follow-up of a randomized clinical trial. *Br J Haematol* 2000;110:577-83.
  14. Harrison CN, Campbell PJ, Buck G, et al. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. *N Engl J Med* 2005;353:33-45.
  15. Campbell PJ, Scott LM, Buck G, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet* 2005;366:1945-53.
  16. Gisslinger H, Kralovics R, Gotic M, Holowiecki J, Penka M, Widmann R et al. Non-inferiority of anagrelide compared to hydroxyurea in newly diagnosed patients with essential thrombocythemia. The ANAHYDRET-Study. *Blood* 2007;110:3547 (abstr.).
  17. Berk PD, Wasserman LR, Fruchtman SM, Goldberg JD. Treatment of polycythemia vera: a summary of clinical trials conducted by the Polycythemia Vera Study Group. In: *Polycythemia Vera and the Myeloproliferative Disorders*, Wasserman LR, Berk PD and Berlin NI eds. WB Saunders, Philadelphia 1995;166-94.
  18. Berk PD, Goldberg JD, Donovan PB, et al. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. *Semin Hematol* 1986;23:132-43.
  19. Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. *J Clin Oncol* 2005;23:2224-32.
  20. Di Nisio M, Barbui T, Di Gennaro L, Borrelli G, Finazzi G, Landolfi R et al. The hematocrit and platelet target in polycythemia vera. *Br J Haematol* 2007;136:249-59.
  21. Tefferi A. New insights into the pathogenesis and drug treatment of myelofibrosis. *Curr Opin Hematol* 2006;13: 87-92.
  22. Tefferi A, Barosi G, Mesa RA et al. International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia for the IWG for Myelofibrosis Research and Treatment (IWG-MRT). *Blood* 2006;108:1497-503.
  23. Sirhan S, Lasho TL, Hanson CA, et al. The presence of JAK2V617F in primary myelofibrosis or its allele burden in polycythemia vera predicts chemosensitivity to hydroxyurea. *Am J Hematol* 2008;83:363-5.
  24. Barosi G, Besses C, Birgegard G, et al. A unified definition of clinical resistance/intolerance to hydroxyurea in essential thrombocythemia: results of a consensus process by an International Working Group. *Leukemia* 2007;21:277-80.
  25. Finazzi G, Barbui T. How I treat patients with polycythemia vera. *Blood* 2007;109:5104-11.
  26. Najean Y, Rain JD for the French Polycythemia Study Group. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. *Blood* 1997;90:3370-7.
  27. Finazzi G, Ruggeri M, Rodeghiero F, Barbui T. Efficacy and safety of long-term use of hydroxyurea in young patients with essential thrombocythemia and a high risk of thrombosis. *Blood* 2003;101:3749.
  28. Finazzi G, Caruso V, Marchioli R, et al. Acute leukemia in polycythemia vera. An analysis of 1,638 patients enrolled in a prospective observational study. *Blood* 2005;105:2664-70.
  29. Sterkers Y, Preudhomme C, Lai J-L, et al. Acute myeloid leukemia and myelodysplastic syndromes following essential thrombocythemia treated with hydroxyurea: high proportion of cases with 17p deletion. *Blood* 1998; 91:616-22.
  30. Murphy S, Iland H, Rosenthal D et al. Essential thrombocythemia: an interim report from the Polycythemia Vera Study Group. *Semin Hematol* 1986;23:177.
  31. Bernasconi P, Boni M, Cavigliano PM, et al. Acute myeloid leukemia (AML) having evolved from essential thrombocythemia (ET): distinctive chromosomal abnormalities in patients treated with pipobroman or hydroxyurea. *Leukemia* 2002;16:2078-83.